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Lolia aquatica gen. et sp. nov. (*Lindgomycetaceae*, *Pleosporales*), a new coelomycete from freshwater habitats in Egypt

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Abstract — An unknown coelomycete that was collected from the River Nile and associated irrigation canals in Egypt is described. The fungus is characterized by gelatinous pearl white acervuli, a peridium that forms textura intricata, holoblastic conidia that have one basal excentric cellular appendage, and up to 3–5 sub-apical cellular attenuating appendages. Based on morphology, no described genus can accommodate this new fungus, so it is described herein as new genus and species. Phylogenetic analyses of the 28S ribosomal large subunit (LSU) rDNA sequence placed the new fungus in the family *Lindgomycetaceae*, *Pleosporales*, *Dothideomycetes*.

Key words — aquatic fungi, anamorphic fungi, subtropical, appendaged conidia

Introduction

Over 7000 coelomycetes in 1000 genera (+ 500 syn.) have been described (Kirk et al. 2008) from a wide range of substrates and geographical locations (Sutton 1980, Nag Raj 1993). A small number of coelomycetes have been linked to their teleomorphs, with affinities to ascomycetes, while a few are basidiomycetes (Nag Raj 1978, 1980, Dyko & Sutton 1979, Cole & Samson 1979, Nag Raj et al. 1989, Rungjindamai et al. 2008). Coelomycetes are a major group of the aquatic mycota of *Phragmites australis* (Van Ryckegem & Verbeken 2005a,b, 2007; Abdel-Aziz 2008). During an investigation of aquatic fungi in Egypt an unknown coelomycete with gelatinous pearl white acervuli was recorded from different localities at the River Nile and irrigation canals in Upper Egypt. This fungus is unique in that it possesses one excentric basal and three to five sub-apical un-branched cellular appendages of type A (Nag Raj 1993). This newly

discovered taxon is described, illustrated, and compared to other appendaged coelomycetes. In addition, we used phylogenetic analyses of the LSU gene to determine its phylogenetic relationship.

Materials and methods

Collection of the fungi

Submerged decayed wood was collected from the River Nile and irrigation canals from Sohag, Qena, and Aswan governorates. Samples were kept in clean plastic bags and returned to the laboratory, examined immediately under stereomicroscope for fungal fruiting structures and subsequently incubated on moist filter paper in sterile plastic boxes. Material was examined periodically over three month's incubation. Single spore isolates of the new fungus were obtained. Photographs were taken using an Olympus BX51 differential interference contrast light microscope and Olympus DP12 digital imaging system (Olympus Corporation, Tokyo, Japan). Herbarium material was dried at 60°C for 24 h and deposited along with the isolated fungal cultures in the authors' culture collection, Department of Botany, Faculty of Science, Sohag University, Egypt. Voucher slides and type material of the new fungus were deposited at International Mycological Institute (IMI).

DNA extraction, sequencing, and phylogenetic analysis

Single-spore isolate of the fungus was grown in YMG broth (4 g yeast extract, 10 g glucose, 10 g malt extract in 1 liter distilled water) until sufficient mycelium had formed to allow DNA extraction. DNA extraction for polymerase chain reaction (PCR) was performed using the Microbial DNA Extraction Kit (MOBIO; Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Partial LSU ribosomal DNA was amplified using primers LR0R and LR7 (Bunyard et al. 1994). PCR reactions, cycling parameters and sequencing were carried out as described by Abdel-Wahab et al. (2009). Sequences were assembled using Sequencher 4.2.2 (Gene Codes Corporation). Sequences were aligned with others retrieved from GenBank using ClustalX (Thompson et al. 1997) and optimized manually. The positions where one or more species contained a length mutation and ambiguously aligned regions were not included in the subsequent phylogenetic analysis. Nucleotide sequence phylogenies were constructed using PAUP* 4.0b10 (Swofford 2002). Maximum-likelihood (ML) analyses (Felsenstein 1981) were performed using heuristic searches with the random stepwise addition of 100 replicates and tree bisection-reconnection (TBR) rearrangements. The optimal model of nucleotide substitution for the ML analyses was determined using hierarchical likelihood ratio tests as implemented in Modeltest 3.7 (Posada and Crandall 1998). The model selected as the best fit for LSU rDNA data set was TrN+I+G. For the bootstrap analyses (Felsenstein 1985), 100 replicates were generated with 5 random additions and TBR. Maximum-parsimony (MP) trees were obtained by 100 random addition heuristic search replicates using PAUP, and 1000 bootstrap replicates were performed employing 5 random addition heuristic searches. Posteriori probability values were obtained by using the MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) with the SYM+I+G model that was determined using MrModeltest 2.2 (Nylander 2004). Five million generations were run in four chains with sampling every 100 generations,

yielding 50 000 trees, of which the first 12 500 were discarded as "burn in." The numbers on the branches are estimates of a posteriori probabilities. The LSU sequence of *Lolia aquatica* isolate used in this study was deposited at GenBank under the accession number "HM367732"; ex-type: MF 644 (JAMSTEC, Japan).

Results

Phylogenetic analyses

The partial LSU rDNA sequence of *Lolia aquatica* is aligned with representatives of the family *Lindgomycetaceae* along with representatives of the families belong to orders *Pleosporales* and *Jahnulales*. In total, the LSU rDNA dataset include 39 taxa of which 2 belong to the class *Pezizomycetes* that

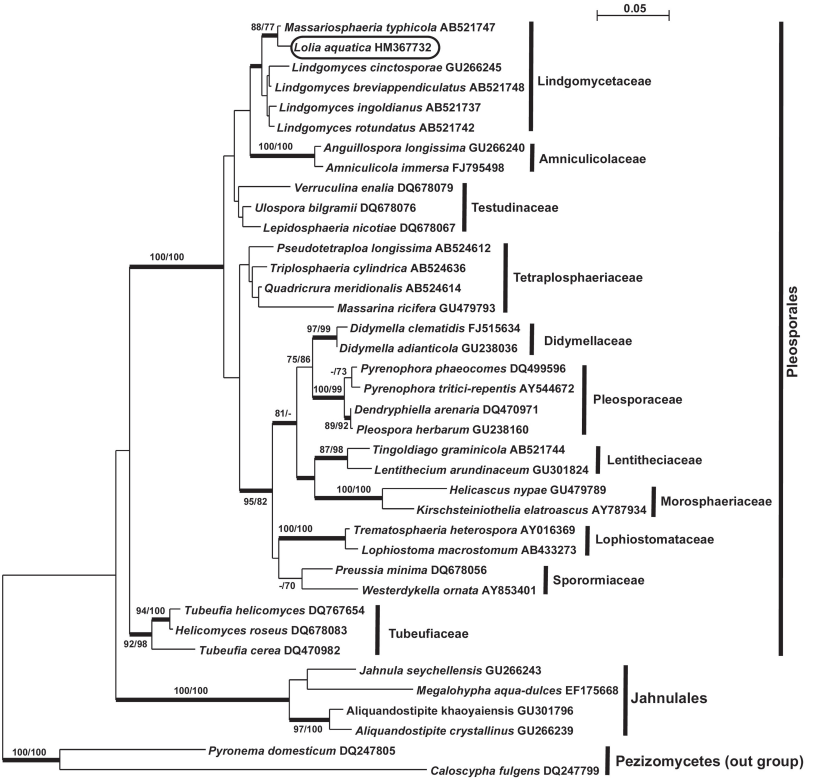


Fig. 1: Phylogenetic relationships of *Lolia aquatica* and closely similar fungi, based on the nucleotide sequences of the large subunit (LSU) rDNA. The maximum likelihood tree (ML) (–ln likelihood = 4978.2339) was constructed as described in the text. The numbers indicate pp values ≥ 95% (in bold), ML bootstrap and MP bootstrap values ≥ 70%. The new species, *Lolia aquatica*, is highlighted in a box.

was used as outgroup. The dataset consisted of 795 total characters, of which 42 gaps are excluded, 477 characters were constant, 69 variable characters were parsimony-uninformative and 207 were parsimony informative characters. Five most parsimonious trees were produced using heuristic search, the five trees have equal length of 817 steps, a consistency index of 0.5141, a retention index of 0.6953 and a rescaled consistency index of 0.3574. Maximum likelihood analysis produced one tree with –ln likelihood score of 4,978.2339 (Fig. 1). Most parsimonious (MP), and Neighbor-Joining (NJ) and Bayesian analyses produced similar trees to the one shown in Fig. 1.

Lolia aquatica is a sister taxon to *Massariosphaeria typhicola* (P. Karst.) Leuchtm. and forms a well supported clade (100/88/77 for Bayesian/ML/MP respectively) within the recently published freshwater ascomycete family, *Lindgomycetaceae* K. Hiray et al. (Schoch et al. 2009, Shearer et al. 2009, Hirayama et al. 2010).

Taxonomy

Lolia Abdel-Aziz & Abdel-Wahab, **anam. gen. nov.**

MycoBank MB 518528

Conidiomata acervularia, margariticoloria, in gelatina immerse, superficialia, solitaria vel gregaria. Peridium ex textura intricata formatum, hyalinum, in matrice gelatinosa immersum. Conidiogenesis holoblastica. Conidia aseptata, clavata, cylindrica vel ellipsoidea, hyalina, levia, tenuitunicata, ad apicem 3–5 appendicibus, ad basim appendice singulari excentrica (typi A).

Type species: *Lolia aquatica* Abdel-Aziz & Abdel-Wahab

Etymology: From the Arabic word, *Loli* = pearl, in reference to the color of the conidiomata.

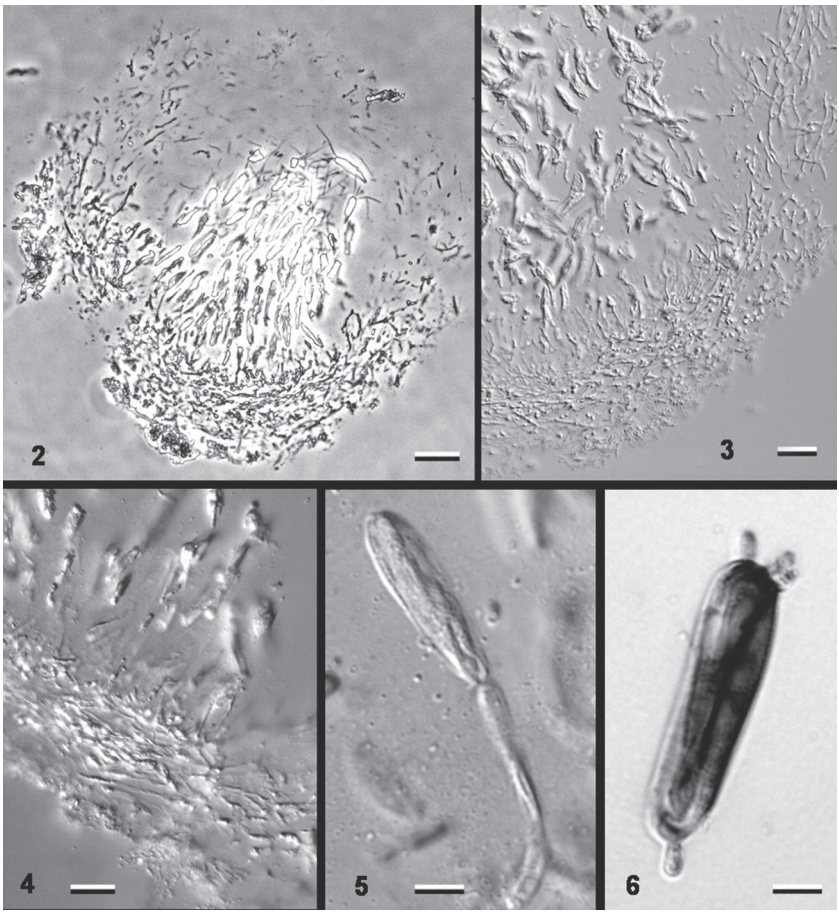
Conidiomata acervular, superficial, pearl white, embedded in gel, single or aggregated. Peridium forming textura intricata, hyaline, embedded in gel. Conidiogenesis holoblastic. Conidia unicellular, clavate, cylindrical, ellipsoidal, hyaline, smooth, thin-walled, with basal and apical cellular, tapering, attenuating appendages of type A.

Lolia aquatica Abdel-Aziz & Abdel-Wahab, **sp. nov.** Figs 2–10

MycoBank MB518529

Conidiomata acervularia, 400–480 µm alta, 380–540 µm diam., margariticoloria, superficialia, solitaria vel gregaria. Peridium 57–80 µm crassum, ex textura intricata formatum, hyalinum, in matrice gelatinosa immersum. Conidiogenesis holoblastica. Conidia 31–45 × 7–10 µm, aseptata, hyalina, clavata, ellipsoidea vel cylindrical, 3–5 appendicibus apicalibus, 55–90 × 1.5–3 µm, et appendice basali singulari, simplici, excentrica, 10–85 × 1.5–3 µm.

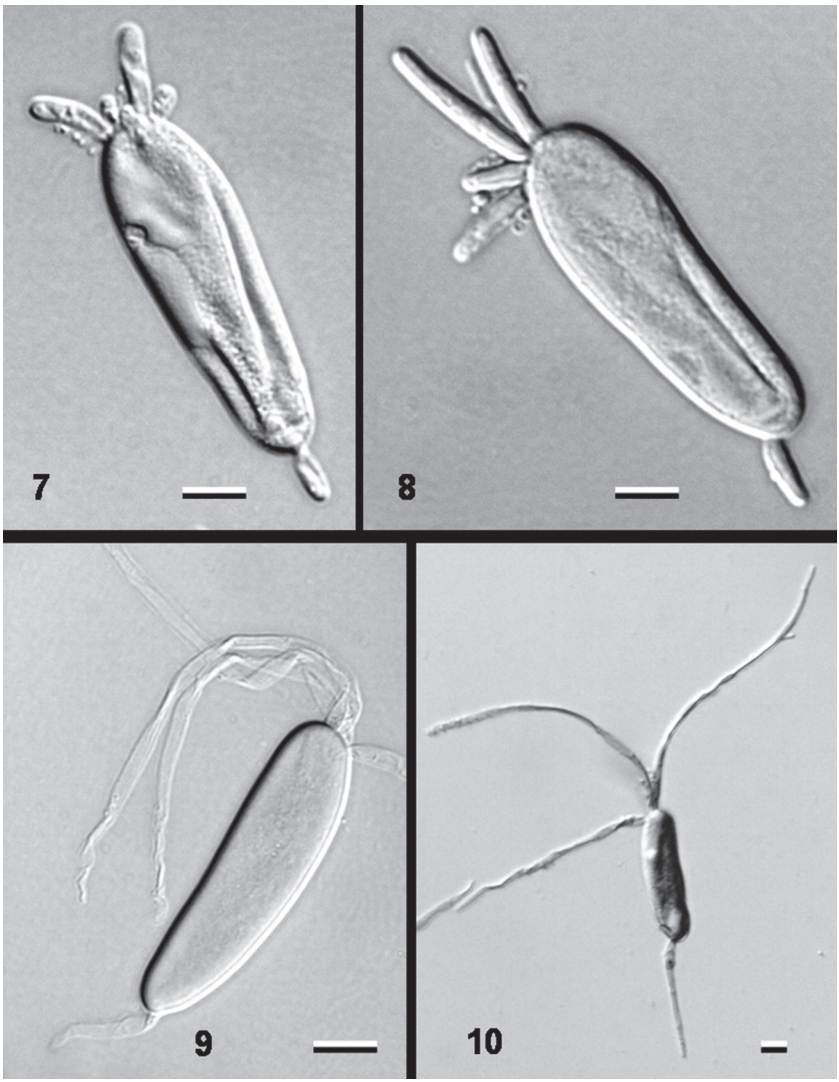
Type: Egypt, Sohag, El Balyana city, on decayed stem of *Phragmites australis* (Cav.) Steud. at irrigation canal, March 2005, F.A. Abdel-Aziz (**Holotype**, IMI 398675; ex-type culture, MF644 (JAMSTEC, Japan); iso-type, MD644 (authors' culture collection).



Figs 2–6: *Lolia aquatica*. Differential interference contrast light micrographs (from holotype, mounted in water). 2. Vertical section through the gelatinous acervular (in phase contrast). 3-4. Magnified part of the peridial wall that forms textura intricata. 5. Young developing conidium at the tip of the conidiogenous cell. 6. Young conidium stained in toluidine blue shows initials of apical and basal appendages. Bars: 2 = 40 µm, 3–4 = 20 µm, 5–6 = 5 µm.

Etymology: From the Latin adjective *aquaticus*, in reference to the freshwater habitat of the fungus.

Conidiomata acervular, 400–480 µm high, 380–540 µm diam, pearl white when wet, dull yellow brown when dry, superficial, single or aggregated (Fig. 2). Peridium 57–80 µm thick, forming textura intricata, hyaline, embedded in gel (Figs 3–4). Conidiophores lining the acervuli wall and arising from innermost elements of the wall, loosely aggregated, branched and septate, colorless, smooth, embedded in gel. Conidiogenous cells cylindrical to sub-cylindrical,



Figs 7–10: *Lolia aquatica*. Differential interference contrast light micrographs of conidia at different stages of development. 10. Stained in toluidine blue. Bars: 7–10 = 5 µm.

colorless, smooth, bearing a single terminal conidium. Conidiogenesis: ontogeny holoblastic with apical wall building; delimitation by a transverse septum; secession schizolytic (Fig. 5). Conidia 31–45 × 7–10 µm (mean = 36 × 8.6 µm, n = 50), unicellular, hyaline, clavate, ellipsoidal, cylindrical, hyaline, smooth, thin-walled, solitary. Mean conidium length/width ratio = 4.2:1. Apical

appendages 55–90 × 1.5–3 µm (mean = 68.6 × 2.6 µm, n = 20), three to five sub-apical cellular appendages, attenuating, tapering. Basal appendage 10–85 × 1.5–3 µm (mean = 27.9 × 2.3 µm), excentric, cellular, attenuating, tapering. Both apical and basal appendages are on one side of the conidia and arising as tubular extension of the conidium body and not separated from it at maturity by septa (Figs 6–10).

Discussion

Several groups of anamorphic fungi are present in freshwater habitats (Shearer et al. 2004, 2007). The best-known and the most studied group is the "aquatic" or "Ingoldian" hyphomycetes, which are distinguished by their tetraradiate, branched, or sigmoid conidia that are released into and dispersed by water (Ingold 1975, Webster & Descals 1981, Bärlocher 1992). About 300 species of aquatic hyphomycetes have been described thus far (Bärlocher 1992, Shearer et al. 2007). The "aeroaquatic hyphomycetes," whose conidia are modified in a variety of ways to trap air for flotation, comprise a second group of anamorphic fungi (Fisher 1979, Michaelides & Kendrick 1982, Webster & Descals 1981, Premdas & Kendrick 1991). Coelomycetes are encountered regularly on a wide variety of submerged plant substrata in both lentic and lotic habitats (Shearer et al. 2004).

Phylogenetic analyses of partial 28S rDNA of *Lolia aquatica* show that it is a member of *Lindgomycetaceae*, *Pleosporales*. Phylogenetically, there are four major exclusive freshwater clades in the *Dothideomycetes* (Schoch et al. 2009), namely, the order *Jahnulales* (Pang et al. 2002, Campbell et al. 2007) and three recently described families: *Lindgomycetaceae*, *Amniculicolaceae* and *Lentitheciaceae* (Schoch et al. 2009, Shearer et al. 2009, Zhang et al. 2009, Hirayama et al. 2010).

There are several coelomycetous genera with hyaline, unicellular, appendages conidia that are somewhat similar to *Lolia aquatica,* e.g., *Chaetospermum* Sacc., *Giulia* Tassi, and *Mycotribulus* Nag Raj & W.B. Kend. *Lolia aquatica* is strikingly similar to *Chaetospermum* species, both having pearl white conidiomata, heavily gelatinized walls that consist of textura intricata, and conidia bearing type A appendages. However *Chaetospermum* species differ in having stromatic, pycnoid conidiomata, and an equal number of conidial appendages (3 to 6) at each end (Sutton 1980, Nag Raj 1993). Phylogenetic analyses of SSU and LSU rDNA placed *Chaetospermum* in the *Basidiomycota* (*Sebacinaceae*; Rungjindamai et al. 2008), whereas *L. aquatica* is in the *Ascomycota*.

The genus *Giulia* has dark-brown to black, immersed pycnidia, conidia bearing apical extra-cellular type D appendages arising by differential gelatinization of the conidium sheath. *Mycotribulus* has immersed to erumpent, brown pycnidia, filamentous paraphyses, and conidia bearing type A appendages at both sides

(one apical centric single appendage and 2-4 lateral basal appendages slightly above the truncate base). Phylogenetic analyses of SSU and LSU rDNA placed *Giulia* and *Mycotribulus* in the *Basidiomycota* (*Corticiaceae* and *Physalacriaceae,* respectively; Rungjindamai et al. 2008).

There are several coelomycetous genera with septate hyaline or colored conidia with cellular apical and basal appendages: e.g., *Bartalinia* Tassi, *Discostroma* Clem., *Discosia* Lib., *Monochaetia* (Sacc.) Allesch., *Pestalotia* De Not., *Pestalotiopsis* Steyaert, *Seimatosporium* Corda, *Seiridium* Nees, *Truncatella* Steyaert. Phylogenetic analyses of LSU rDNA placed all the above-mentioned genera in the family *Amphisphaeriaceae*, *Xylariales* (Jeewon et al. 2002).

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